DESCRIPTION

PROTEIN COMPLEX, PROCESS FOR PRODUCING THE SAME AND USE THEREOF

Technical Field

This invention relates to a protein complex, a process for producing the same, and use of the protein complex in a biosensor, an immobilized enzyme and so on.

Background Art

Conventionally, a so-called protein complex, in which a protein is encapsulated in another protein, has been known. As for production of this type of protein complex, for example, a method of applying a solution of a dissolved protein to a surface of a crystalline protein is considered.

However, it is extremely difficult to carry out this method without dissolving the crystalline protein. Accordingly, the fact is that this method is hardly adopted for the purpose of protecting a useful protein (hereinafter referred to as a target protein) such as an enzyme, an antigen, an antibody, a cytokine or a receptor.

As for protection of a target protein, a method of covalently binding a polymer such as a polysaccharide polymer or polyethylene glycol to a target protein has been adopted. This method is a method in which a polymer is bound to a

functional group such as an amino group or a carbonyl group in the target protein under mild reaction conditions. However, in this method, the binding site, the catalyzed site or the like of the target protein could not be controlled. In addition, since the binding site, the catalyzed site or the like varies depending on the type of the target protein, the method could not be applied to all the target proteins.

As for preservation of a target protein, generally, a method of preservation at a lower temperature is employed. In addition, a method of adding or mixing a protective substance (e.g., a polysaccharide polymer, polyethylene glycol and the like), which is expected to have a function of stabilizing the protein structure, to or with a target protein is also employed. However, by employing these methods, the stability or the function of the target protein was lost in some cases due to the changes in the environment, which is an external factor. That is, it is because the target protein is easy to dissolve together with the protective substance when water comes in contact, temperature or humidity increases, or dew condensation occurs. In addition, the target protein is degraded or ingested together with the protective substance when putrefactive bacteria such as germs or fungi exist, penetrate, or emerge. Therefore, when the target protein is a polymeric protein such as a protein molecule of some enzymes or antibodies, it lose its function completely by subjecting

to a change in even a part of its structure or by degrading a part thereof with the action of a protease. However, when the target protein is used, it is essential that it sufficiently have its function. Therefore, it is necessary to verify the stability of the target proteins in a state of preservation individually. In the case of employing a conventional technique, it is necessary to take the target protein out of the protective substance, therefore, not only it takes a lot of time and efforts, but also the target protein is susceptible to denaturation.

By the way, cytoplasmic polyhedrosis virus forms a polyhedron composed of a polyhedral protein in a cell infected with the virus during the late phase of the viral infection, and many virus particles are embedded in the polyhedron.

The reason why the virus particles enter specifically in this polyhedron is known and it is due to the specific relationship between a capsid protein VP3 of the virus particle and a polyhedral protein (Non-Patent Document 1).

In view of the above-mentioned background, the present inventor completed the invention, which relates to a protein complex contributing to protection, preservation and improvement in stability of a target protein and a process for producing the same, and applied for a patent previously (Patent Document 1). The object of the description of the above-mentioned invention is to embed a polymeric target

protein in this polyhedron and to enhance the embedding efficiency. Therefore, by shortening a gene encoding a capsid protein of cytoplasmic polyhedrosis virus, the size (molecular weight) of a protein which can be embedded in a polyhedron is made large, and this target protein is further more efficiently embedded in the polyhedron. Further, as the method, the amino acid sequence of VP3, which is a constituent protein of the envelope of cytoplasmic polyhedrosis virus, is introduced to the N-terminus or the C-terminus of the target protein, and this fusion protein is expressed with a baculovirus vector. At this time, by infecting an insect cell together with a virus expressing a polyhedral protein of cytoplasmic polyhedrosis virus, the fusion protein is embedded in a polyhedron. Accordingly, it is necessary to fuse a cDNA encoding a constituent protein of cytoplasmic polyhedrosis virus and a gene encoding a target protein so that a foreign protein expressed with a baculovirus vector, namely, a target protein is inserted at the N-terminus or the C-terminus of the constituent protein of cytoplasmic polyhedrosis virus. At this time, it is important that the open reading frames encoding the constituent protein and the protein of the target protein gene are cloned in-frame. In this way, a recombinant baculovirus expressing the constituent protein of cytoplasmic polyhedrosis virus and the target protein as one fusion protein is constructed, which is described in the above-mentioned

invention.

Patent Document 1: International Patent Application WO 02/36785A1

Non-Patent Document 1: Ikeda et al., (2001) J. Virol. 75, 988-995

Disclosure of the invention

The present invention is completed by further improving the above-mentioned invention and identifying VP3, which is used as an embedding signal for polyhedron, within the specific area.

An object of the present invention is to provide a protein complex that can encapsulate a target protein whose size (molecular weight) is increased, in addition a target protein having a fluorescent or light-emitting function or a bioactive function, and moreover a polymeric target protein, and further can verify the function of the target protein in a state of a complex.

In addition, another object of the present invention is to provide a production process that can efficiently produce a protein complex having any of target proteins with a variety of properties encapsulated therein without lowering the function thereof.

Further, another object of the present invention is to provide use of a protein complex in a biosensor, an immobilized

enzyme and so on.

A gist of the present invention is a protein complex comprising a polyhedral protein having an insect virus encapsulated therein and a target protein having a restricted region of a capsid protein VP3 of cytoplasmic polyhedrosis virus as an embedding signal for polyhedron.

The restricted region of VP3 is either a region from the N-terminus to the 40th amino acid residue or the region from the 41st amino acid residue to the 79th amino acid residue. In this case, a gist of the present invention is a protein complex comprising a polyhedral protein having an insect virus encapsulated therein and a target protein having, as an embedding signal for polyhedron, a restricted region, which is either a region from the N-terminus to the 40th amino acid residue or a region from the 41st amino acid residue to the 79th amino acid residue of a capsid protein VP3 of cytoplasmic polyhedrosis virus.

The polyhedral protein has an effect on improvement in the stability of the target protein, protection thereof or improvement in the preservation property thereof, or a combination of any of these. In this case, a gist of the present invention is a protein complex comprising a polyhedral protein having an insect virus encapsulated therein and a target protein having, as an embedding signal for polyhedron, a restricted region of a capsid protein of cytoplasmic

polyhedrosis virus, more specifically, a restricted region, which is either a region from the N-terminus to the 40th amino acid residue or a region from the 41st amino acid residue to the 79th amino acid residue of VP3, in which the polyhedral protein has an effect on improvement in the stability of the target protein, protection thereof or improvement in the preservation property thereof, or a combination of any of these.

The target protein is at least one member selected from the group consisting of fluorescent or light-emitting proteins, enzymes, antigens, antibodies, cytokines, receptors and bioactive proteins. In this case, a gist of the present invention is a protein complex comprising a polyhedral protein having an insect virus encapsulated therein and a target protein having, as an embedding signal for polyhedron, a restricted region of a capsid protein of cytoplasmic polyhedrosis virus, more specifically, a restricted region, which is either a region from the N-terminus to the 40th amino acid residue or a region from the 41st amino acid residue to the 79th amino acid residue of VP3, and being at least one member selected from the group consisting of fluorescent or light-emitting proteins, enzymes, antigens, antibodies, cytokines, receptors and bioactive proteins, in which the polyhedral protein preferably has an effect on improvement in the stability of the target protein, protection thereof or

improvement in the preservation property thereof, or a combination of any of these.

In addition, a gist of the present invention is a process for producing a protein complex, wherein a cell is infected with a vector that has been integrated with a gene encoding a target protein together with a vector that has been integrated with a gene encoding a polyhedral protein, and the cell is cultured, whereby a protein complex having a complex structure composed of the target protein and the polyhedral protein is produced in the cell.

Still further, a gist of the present invention is a biosensor characterized in that a protein complex comprising a polyhedral protein having an insect virus encapsulated therein and a target protein having, as an embedding signal for polyhedron, a restricted region of a capsid protein of cytoplasmic polyhedrosis virus, more specifically, a restricted region, which is either a region from the N-terminus to the 40th amino acid residue or a region from the 41st amino acid residue to the 79th amino acid residue of VP3, and more specifically, being at least one member selected from the group consisting of fluorescent or light-emitting proteins, enzymes, antigens, antibodies, cytokines, receptors and bioactive proteins, in which the polyhedral protein preferably has an effect on improvement in the stability of the target protein, protection thereof or improvement in the preservation property

thereof, or a combination of any of these, is arranged in dots or lines on a substrate and immobilized thereon, a biosensor characterized in that the protein complex is packed in such a manner that it can be contacted with a substance in a test solution in a recess formed on a substrate, or a biosensor characterized in that the protein complex is packed in a container in such a manner that it can be contacted with a substance in a test solution.

In addition, a gist of the present invention is an immobilized enzyme in which a protein complex comprising a polyhedral protein having an insect virus encapsulated therein and a target protein having, as an embedding signal for polyhedron, a restricted region of a capsid protein of cytoplasmic polyhedrosis virus, more specifically, a restricted region, which is either a region from the N-terminus to the 40th amino acid residue or a region from the 41st amino acid residue to the 79th amino acid residue of VP3, and more specifically, being at least one member selected from the group consisting of fluorescent or light-emitting proteins, enzymes, antigens, antibodies, cytokines, receptors and bioactive proteins, in which the polyhedral protein preferably has an effect on improvement in the stability of the target protein, protection thereof or improvement in the preservation property thereof, is packed in a container.

Brief Description of the Drawings

Fig. 1 is a figure illustrating a method of shortening VP3 gene and preparation of a transfer vector.

Fig. 2 is a figure showing the relationship between a shortened VP3 gene and the amino acid residues encoded by the gene.

Fig. 3 shows the determination whether or not a protein encoded by a gene, in which a shortened VP3 gene has been introduced into an EGFP gene, is encapsulated in a polyhedron based on the presence or absence of green fluorescence from the polyhedron.

Fig. 4 shows a green fluorescence intensity observed in a state where EGFP having the nucleotide sequence of a shortened VP3 at the N-terminus is encapsulated in a polyhedron. The fluorescence intensity was graded at five levels, 1+, 2+, 3+, 4+ and 5+.

Fig. 5 shows the results obtained by introducing the region from the 39th amino acid residue to the 79th amino acid residue of VP3 into the N-terminus of Cyclin-dependent kinase 5 as a signal for encapsulation in a polyhedron, thereby encapsulating this protein in a polyhedron, and attaching the polyhedron to a slide glass and performing an antigen-antibody reaction on the surface of the polyhedron.

Best Mode for Carrying Out the Invention

A protein complex according to the present invention comprises a target protein having a restricted region of a capsid protein VP3 of cytoplasmic polyhedrosis virus as an embedding signal for polyhedron encapsulated in a polyhedral protein having an insect virus encapsulated therein. Here, "encapsulation" means that it includes a state where a target protein is completely encapsulated in the inside of a polyhedral protein and a state where it is embedded while a part thereof is exposed to the outside of the polyhedral protein. In addition, examples of the shape of the complex include a regular shape such as cube, rectangular parallelepiped and cylinder, and an irregular shape such as a particulate form. According to the shape, the amount of the encapsulated target protein can be increased, the size of the target protein can be increased, or a function such as a bioactive function or a catalytic function can be dramatically enhanced.

In the present invention, the restricted region of VP3 is a region from the 41st amino acid residue to the 79th amino acid residue as well as a region from the N-terminus to the 40th amino acid residue. Incidentally, though it takes time and efforts and is inefficient, a region in which 10 amino acid residues have been added to the N-terminus or the C-terminus of a region from the 41st amino acid residue from the N-terminus to the 79th amino acid residue can also be used.

Further, when considering the point of binding to a

biologically related chemical substance, the target protein is an enzyme, an antigen, an antibody, a receptor or a cytokine, when considering the point of a photochemical property, it is a light-emitting protein, and when considering the point of an electron transfer reaction, it is a metal-binding protein or a metal ion-containing enzyme. When considering the point of such a property of a target protein, a constitution which is selected from these and is at least one member is preferred.

A process for producing a protein complex according to the present invention comprises introducing a vector that has been integrated with a DNA encoding a target protein having a restricted region of VP3 as a signal simultaneously or together with a vector that has been integrated with a DNA encoding a polyhedral protein into a cell such as an insect cell, an animal cell, a plant cell or an acellular cell, and culturing the cell under the conditions suitable for each cell. In this way, the protein complex can be efficiently produced without lowering its function. However, the vector that has been integrated with a DNA encoding a target protein and the vector that has been integrated with a DNA encoding a polyhedral protein are a plasmid vector, a virus vector or the like, and it is only necessary to individually select the one suitable for a cell into which a DNA is introduced.

As for use of a protein complex of the present invention, it can be applied as a biosensor such as an immunosensor, a

gene sensor or a lipid sensor by arranging and immobilizing the protein complex on a substrate to use the protein complex as a receptor, converting light amount or mass into an electrical signal by a transducer such as SPR, a photon counter or a crystal oscillator, and displaying the electrical signal. As a material for the substrate, a glass, a plastic, a metal or the like can be used. In addition, as a method of bonding the substrate and the protein polyhedron, an adhesive such as gelatin or a macromolecular polymer can be used.

In addition, by using a tubular container, in which a test solution can be passed through, instead of the above-mentioned substrate, and packing the protein complex in the container in such a manner that it can be contacted with a substance in the test solution, it can be applied as a biosensor.

Further, by preparing a particulate protein complex by the same method as in Example 1 using a DNA encoding an enzyme such as a protease, a lipase or an esterase having a catalytic ability, and packing the protein complex in a container in any of various forms, it can be applies as an immobilized enzyme having a catalytic ability.

The detail of the invention of this application will be described with reference to Examples. However, the invention of this application is by no means limited to these Examples.

Example 1

The present invention will be described according to Examples and the attached figures to explain it in more detail.

(1) Preparation of a virus producing a polyhedral protein

In the case where a polyhedron is produced with IPLB-Sf21-AE (Sf21) derived from an insect cell Spondoptera frugiperda, to form a cubic polyhedron of Bombyx mori cytoplasmic polyhedrosis virus (BmCPV), a recombinant virus (AcCP-H) that had been integrated with a polyhedral protein gene of BmCPV strain H (Mori et al., (1993) J. Gen. Virol. 74, 99-102) was inoculated. This AcCP-H is a recombinant virus that has been integrated with a polyhedral protein gene of strain H at the downstream of the polyhedrin promoter of a baculovirus vector derived from Autographa californica nucleopolyhedrovirus (AcNPV).

(2) Analysis of a signal composed of only a restricted region of a capsid protein VP3

1) Shortening of BmCPV S4 encoding a capsid protein VP3

A plasmid pVP3(XbaI)EGFP (International Patent Application WO 02/36785A1) was digested with a restriction enzyme XbaI, and further digested with a restriction enzyme KpnI. This DNA was dissolved in 100 μl of ExoIII buffer in a tube, and 1 μl of Exonuclease III was added, stirred and

incubated at 25°C. This DNA solution was sampled (5 μl each) at 30 second intervals and added to 100 μl of MB Nuclease Buffer which had been prepared in another tube. The mixture was incubated at 65°C for 5 minutes to inactivate Exonuclease III and cooled down to 37°C again. Then, 2 μl of Mung Bean Nuclease was added and the mixture was incubated at 37°C for 30 minutes. After performing phenol extraction and ethanol precipitation, DNA was dissolved in 50 μl of Klenow Buffer, and 1 μl of Klenow Fragment was added. After the mixture was incubated at 37°C for 15 minutes, thereby completely repairing the ends, 10 μl of the mixture was taken out and added to 100 μl of Ligation Solution A which had been prepared in another tube. Further, 12 μl of Ligation Solution B was added and stirred, and the mixture was reacted at 16°C for 3 hours. Then, ethanol precipitation and rise were carried out. After the collected DNA was digested with a restriction enzyme XbaI for 1 hour, the mixture was added to a competent cell JM109 (100 μl), whereby transformation was carried out. Incidentally, the above-mentioned procedure was carried out by using, for example, Kilo-Sequence Deletion Kit (manufactured by Takara Co.) according to its protocol (Fig. 1).

2) Construction of a recombinant transfer vector

The transformed E. coli was plated on a 2xTY plate containing kanamycin and cultured overnight at 37°C. The

formed colonies were cultured overnight at 37°C in 2xTY medium containing kanamycin. The plasmid DNA was extracted, digested with restriction enzymes BglII and BamHI and electrophoresed. It was confirmed that the DNA fragment was shortened, and a sequence analysis was performed, whereby the nucleotide sequence of the DNA fragment was confirmed. The plasmid DNA solution which was required for confirmation of the nucleotide sequence was digested with a restriction enzyme NotI, and inserted at the NotI site of a baculovirus transfer vector pVL1392 (manufactured by PHARMINGEN). A competent cell JM109 (100 μl) was transformed with this vector, plated on a 2xTY plate containing ampicillin and cultured overnight at 37°C. The formed colonies were cultured overnight at 37°C in 2xTY medium containing ampicillin. The plasmid DNA was extracted, and a sequence analysis was performed. From the results of the analysis, the one in which the insert was inserted in the right direction was selected, which was used as a recombinant transfer vector pAcVP3(x)/EGFP (with the proviso that x represents the number of bases of the S4 cDNA encoding VP3 of BmCPV) (Fig. 2).

3) Construction of a recombinant baculovirus

A cultured insect cell Sf21 was cotransfected with the constructed recombinant transfer vector pAcVP3(x)/EGFP (5 μg each) and a linear Baculogold Baculovirus DNA (0.5 μg)

(manufactured by PHARMINGEN) according to the lipofectin method. Subsequently, the plaque was purified, whereby a recombinant virus AcVP3(x)/EGFP was constructed.

(3) Preparation of a protein complex containing EGFP as a target protein

1) Expression of the recombinant protein in Sf21 cell

As a control, double infection with AcVP3/GFP (Ikeda et al., (2001) J. Virol. 75, 988-995) and AcCP-H (Mori et al., (1993) J. Gen. Virol. 74, 99-102) or with AcVP3(XbaI)/ GFP (International Application WO 02/36785A1) and AcCP-H, was performed. On the other hand, for the purpose of shortening VP3, double infection with AcVP3(x)/GFP and AcCP-H was performed. The double infection was performed at 10 p.f.u./cell for each case. After the virus was allowed to adsorb to cells at room temperature for 1 hour, the virus solution was removed, and 2 ml of TC-100 containing 10% fetal bovine calf serum was added, and the mixture was incubated at 27°C for 4 days.

2) Purification of polyhedra

The cubic polyhedra were collected from the infected cells on the 4th day. After washing with PBS (20 mM NaH2PO4, 20 mM Na2HPO4, 150 mM NaCl, pH7.2), the polyhedra were homogenized in ice with a homogenizer. The homogenate was

washed with 1% Tween 20, and the polyhedra were collected by centrifugation. Then, centrifugation with the sucrose density gradient from 1.5 M to 2.2 M at 50,000 x g for 45 minutes was performed to extract the fraction of polyhedra. The extracted sample was washed with PBS, followed by centrifugation at 15,000 x g for 10 minutes, and purified polyhedra were collected.

3) Determination of encapsulation of EGFP in a polyhedron

Polyhedra from cells subjected to double infection with AcVP3(X)/GFP and AcCP-H, and as a control, AcVP3/GFP and AcCP-H, and AcVP3(XbaI)/GFP and AcCP-H were purified, and encapsulation of EGFP in a polyhedron was determined based on the presence or absence of fluorescence from the polyhedron using a fluorescence microscope (manufactured by OLYMPUS-IX71) (Fig. 3). As a result, in any case, green fluorescence from the polyhedron was observed, and it was confirmed that VP3/GFP or VP3(XbaI)/GFP was encapsulated in the polyhedron.

Subsequently, for all AcVP3(X)/GFP prepared as shown in Fig. 2, encapsulation of EGFP in the polyhedron was investigated. As a result, it was found that a VP3(250)/GFP molecule encoded by a chimeric gene in which a region containing from the 5'-terminus to the 250th base of the VP3 gene had been introduced into the 5'-terminus of the EGFP gene was embedded

in the polyhedron. That is, it means that a signal for embedding a protein molecule specifically in the polyhedron (embedding signal for polyhedron) exists in a region up to the 79th amino acid residue at the N-terminus of VP3. Because of the existence of this signal, a VP3(250)/GFP molecule is encapsulated in the polyhedron , and as a result, green fluorescence from the polyhedron could be observed as shown in Fig. 3.

However, in the case of a chimeric gene in which a region containing from the 5'-terminus to the 130th base of the VP3 gene had been introduced into the 5'-terminus of the EGFP gene, a fusion GFP molecule VP3(130)/GFP encoded by this chimeric gene lost the function of being encapsulated in the polyhedron, and green fluorescence from the polyhedron was not observed at all (Fig. 3). This indicates that the embedding signal for polyhedron does not exist in the region up to the 39th amino acid residue at the N-terminus of VP3.

Further, a fragment from the 135th base to the 292nd base of VP3 was amplified by the PCR method, and a chimeric gene in which the amplified fragment was introduced into the 5'-terminus of the EGFP gene was constructed. As a result, a region encoding from the 41st amino acid residue at the N-terminus to the 93rd amino acid residue of VP3 is introduced into the N-terminus of EGFP. It was confirmed whether this VP3(135-292)/EGFP was encapsulated in the polyhedron in a

similar manner, as a result, green fluorescence from the polyhedron was observed.

From the above result, for embedding of a protein molecule in a polyhedron via VP3, a very limited N-terminal region of VP3, that is, a region from the 41st amino acid residue at the N-terminus to the 79th amino acid residue of VP3 is found to function as an embedding signal for polyhedron.

Effect of shortening of VP3

By introducing a gene encoding a region with different length from the 5'-terminus of the VP3 gene into the 5'-terminus of the GFP gene, regions of various amino acid sequences derived from VP3 were introduced into the N-terminus of GFP. The color development of green fluorescence by a fusion GFP molecule expressed from any of these chimeric genes was compared. As a result, as shown in Fig. 4, as the region of VP3 to be introduced into the N-terminus of GFP became shorter, the color development of green fluorescence was increased. However, in the case where the region was made shorter than the 79th amino acid residue from the N-terminus of VP3, the color development of green fluorescence was substantially the same. In this way, in the case where another amino acid sequence is introduced into a target protein, as the length of the sequence becomes shorter, the bioactivity of the target protein is increased. However, the sequence becomes shorter than necessary, the

function as the signal will be lost.

The signal for encapsulating a target protein in the polyhedron of VP3 obtained in the present invention has a function sufficient for encapsulating the target protein in the polyhedron when it was introduced in the target protein molecule. Moreover, the signal has a length that does not disturb the bioactivity of the target protein. Further, it is indicated that by shortening the length of VP3 according to the present invention, a molecule which is larger by the length of VP3 that had been removed can be embedded in the polyhedron, therefore, the effect of the present invention is high.

Subsequently, according to the procedure of Example 1, a biosensor using a cubic protein complex about 10 μm on a side by applying human-derived Cyclin-dependent kinase (CDK5) as a target protein will be explained.

Example 2

A biosensor was prepared by arranging a complex on a slide glass.

On a slide glass, 5 μl of a gelatin solution (gelatin: 0.5, Crk: 0.02) was dropped. Incidentally, Crk is chromium potassium sulfate (an antiseptic).

The front sides of the slide glass and a new slide glass were put together carefully. When the solution was spread

therebetween, the slide glasses were slowly pulled apart. After the gelatin was completely dried, 1 μl of a complex solution which had been well stirred was dropped thereon, then dried, whereby a biosensor was prepared. This sensor was immersed in distilled water until use.

Incidentally, a complex solution represents a solution obtained by purifying a complex which has been expressed in a large amount in Sf21 cells, and suspending the purified complex in distilled water.

Verification

Verification method

(1) Suppression of peroxidase activity

A hydrogen peroxide solution (adjusted to a final concentration of 1% by PBS) was placed on the part where the complex was dropped. After a 15-minute treatment at room temperature, washing was carried out with PBS (in order to remove the hydrogen peroxide solution).

In this way, the peroxidase activity to be a background was suppressed.

(2) Blocking with normal serum (5% NHS)

Normal horse serum was adjusted to a final concentration of 5% with PBS containing 0.3% Triton X-100 (T-PBS), and added to the slide glass. After a 20-minute treatment at room temperature, washing was carried out with T-PBS.

(3) Primary antibody reaction

An anti-Cdk5 monoclonal antibody was diluted to 100-fold with T-PBS containing 5% serum, and reaction was carried out at 37°C for 3 hours. Then, washing was carried out with T-PBS.

(4) Biotinylated anti-mouse IgG antibody reaction

A biotinylated secondary antibody was diluted to 100-fold with T-PBS, and reaction was carried out at 37°C for 1 hour. Then, washing was carried out with T-PBS.

On the other hand, A solution and B solution to be used in ABC reaction were diluted to 100-fold with T-PBS, and reaction was carried out for at least 30 minutes in advance.

(5) Reaction with ABC reagent (VECTASTAIN ABC KIT STANDARD PK-6100)

After 1-hour reaction at room temperature, washing was carried out with T-PBS.

(6) Washing

Since precipitate is formed by the reaction of the subsequent DAB with phosphoric acid, in order to replace PBS, washing was carried out lightly with 50 mM Tris-HC (pH 7.5), and the solution was replaced.

(7) Incubation with DAB substrate

DAB powder was added to 50 mM Tris-HCI solution at a concentration of 50 mg/ml, 16 μl of hydrogen peroxide solution was further added, and reaction was carried out at room temperature for 25 minutes. After the reaction, the slide

glass was immersed in 50 mM Tris-HCI solution.

(8) Encapsulation with glycerol/PBS

After the slide glass was dried, one drop of glycerol/PBS was dropped on the sample, then a cover glass was placed thereon avoiding any air bubble under.

By using the above-mentioned verification method, an antigen-antibody reaction was attempted with a protein complex having a target protein encapsulated therein and a polyhedron without any protein encapsulated therein. The results are shown in Fig. 5. As shown in Fig. 5, as for the protein complex having Cdk5 encapsulated therein, the antigen-antibody reaction of the Cdk5 molecule and the anti-Cdk5 antibody could be observed on its surface. In this way, an antigen-antibody reaction, that is, a protein-to-protein interaction between an antigen protein and an antibody protein can be observed on the surface of a protein complex having a fused target protein encapsulated therein.

Industrial Applicability

As described in detail above, according to the present invention, a protein complex comprising a polyhedral protein and a target protein can be efficiently produced by introducing a polyhedral protein, which is a constituent protein of a polyhedron having essentially an insect virus encapsulated therein and only a restricted region of a capsid protein VP3

of cytoplasmic polyhedrosis virus as a signal into a target protein.

In addition, a protein complex obtained by encapsulating a protein molecule having a bioactive function such as an enzymatic activity, an antigen- antibody reaction or a protein-to-protein interaction in a polyhedral protein can be used as an excellent biosensor or immobilized enzyme.